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(54) Title: A FAT CELL SPECIFIC β-ADRENERGIC RECEPTOR

(57) Abstract

The present invention relates to a fat cell specific β-adrenergic receptor that mediates lipolysis. The invention further relates to cloned cells which code for the specific β-adrenergic receptor that mediates lipolysis. Another aspect of the present invention relates to a diagnostic test method for determining decreased levels of fat cell β-adrenergic receptors that mediate lipolysis in order to diagnose obesity caused by less active lipolysis.

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A FAT CELL SPECIFIC β-ADRENERGIC RECEPTOR

Technical Field

This application relates to fat cell specific β-adrenergic receptors from brown adipose tissue and clone cells related to the receptor.

Background of the Invention

There has long been an interest in the structure of adipose tissue as it relates to a possible role in obesity. Brown adipose tissue is the main effector of cold- and diet-induced thermogenesis in mammals, such as rodents. See Foster et al., Can. J. Physiol., Vol. 56, 110 (1978) or Rothwell et al., Nature (London), Vol. 281, 31 (1979). The process of thermogenesis can represent a major expenditure of energy and play an important role in overall energy balance. Because brown adipose tissue has been demonstrated in humans of all ages and is often atrophied or quiescent in obese animals, much interest has recently been directed towards development of compounds that stimulate the thermogenesis metabolic response as possible anti-obesity agents.

Brown adipose tissue metabolism is primarily controlled by norepinephrine released from the sympathetic nerve terminals that act through β-adrenergic receptors. Both β_1- and β_2-adrenergic

receptor subtypes are present in rat brown adipose tissue; however, pharmacological studies with novel thermogenic β-adrenergic agonists have suggested the existence of an atypical β-adrenergic receptor in the brown adipose tissue that mediates lipolysis (breakdown of fat). Parallel studies have also suggested the presence of atypical β-adrenergic receptors with similar pharmacological properties in white adipose tissue, the digestive track, and in skeletal muscle.

Accordingly, there is a need in the art for isolation and understanding of the fat cell β receptor or receptors which are related to the thermogenesis process. Such an isolation of the β-adrenergic receptor(s) would allow for the diagnosis of obesity, the treatment of obesity, the testing of medications for their effectiveness in stimulating the thermogenesis metabolic response in obesity patients.

Disclosure of the Invention

An object of the invention relates to obtaining the sequence of a β-adrenergic receptor polypeptide that mediates lipolysis and which is produced by β-adrenergic fat cell receptor clones.

Another object of the present invention is to produce clone cells coding for fat cell β-adrenergic polypeptide receptors that mediate lipolysis.

A further object of the invention is to choose several clonal cell lines that permanently express the fat cell β receptor, which mediate lipolysis, and choose one of the cell lines for additional pharmacological and biochemical characterization.

A further object of the invention is to provide a diagnostic test for determining decreased levels of the fat cell β-adrenergic receptor that mediates lipolysis in order to diagnose obesity caused by less active lipolysis.

Brief Description of the Figures

Figure 1 relates to a comparison of adrenergic receptor polypeptides of humans and rats. This figure shows human β-2, rat β-2, rat β, human β-1, human β-3 and rat β-3 receptor sequences.

Figure 2 relates to the percent of Forskolin-stimulated cAMP production in transfected CHO cells expressing the fat cell β receptor according to the present invention with a rank order of potency of agonists BRL 37344 - isoproterenol - norepinephrine - epinephrine - zinterol - tazolol.

Figure 3 relates to the potency of antagonists (at 10^{-4} M concentrations) for inhibiting BRL 34344-induced cAMP accumulation in transfected CHO cells for several antagonists.

Figure 4 shows the distribution of β-adrenergic receptor sub-types poly(A)$^+$ RNAs from various tissues which were isolated and fractionated on a formaldehyde-agarose gel. The tissues were brown adipose tissue (BAT), white adipose tissue (WAT), brain (Brn), heart (Hrt), ileum (Ile), liver (Liv), or lung (Lng).

Figure 5 compares the level of β_1, β_2 and β_{3A}-adrenergic receptor mRNA levels in brown and white fat of obese rats as compared to lean controls. The dotted line represents 100% as the amount of adrenergic receptor found in the lean rat. The white histogram box represents the β_1 receptor, the diagonally cross-

hatched histogram box represents the β_2 receptor and the darkened histogram box represents the level of β_{3A}-adrenergic receptor mRNA.

Figure 6 relates to a polypeptide having a sequence according to SEQ ID NO:1.

Description of the Invention Preferred Embodiments

The present invention relates to a fat cell specific β-adrenergic receptor that mediates lipolysis. Particularly preferred is a β-adrenergic receptor polypeptide having a sequence according to SEQ ID NO: 1.

The present invention also provides cloned cells encoding for a fat cell β-adrenergic receptor that mediates lipolysis. Further provided is a clone cell which is obtained by cotransfection of CHO cells. More preferred are clone cells which produce a β_{3A}-adrenergic receptor. Even more preferred are clone cells which produce an adrenergic receptor having the sequence according to SEQ ID NO: 1.

The invention still further provides a diagnostic test for determining decreased levels of fat cells β-adrenergic receptors that mediate lipolysis in order to diagnose obesity caused by less active lipolysis. More preferred is a diagnostic test for determining decreased levels of a β-adrenergic receptor polypeptide having a sequence according to SEQ ID NO: 1.

Experimental

A rat interscapular brown adipose tissue (IBAT) cDNA library was cloned and probed with DNA probes encoding human β_1- and rat β_2-adrenergic receptors

under conditions of low stringency. Nine positive clones were identified that were demonstrated by restriction mapping to be different from rat β_1 and β_2 adrenergic receptor cDNAs. Sequence analysis of these clones reveal the presence of a single opening reading frame of 1,200 bp encoding a polypeptide of about 400 amino acids with a predicted size of 43,169 daltons.

The adipose tissue β-adrenergic receptor has 49% and 40% identity, respectively to rat β_1- (C.A. Machida et al, J. Biol. Chem. 265, 12960 (1990)) and β_2-adrenergic receptors (D.A. Robinson, thesis, State University of New York at Buffalo (1988)) and 80% identity to the human β_3-adrenergic receptor (L.J. Emorine et al, Science 245, 1118 (1989)) (Figure 1).

Sequence identity between β_1- and β_2-adrenergic receptors from rats (C.A. Machida et al, J. Biol. Chem. 265, 12960 (1990); D.A. Robinson, Thesis, State University of New York at Buffalo (1988)) and humans (T. Frielle et al, Proc. Natl. Acad. Sci. USA 84, 7920 (1987); F.Z. Chung et al, FEBS Lett. 211, 200 (1987)) is extremely high: 90% for β_1-adrenergic receptors and 87% for β_2-adrenergic receptors.

While the rat adipose tissue β-adrenergic receptor is more closely related to the human β_3-adrenergic receptor than to either rat β_1- or β_2-adrenergic receptor subtypes, the amino acid identity is lower than might be expected for species differences alone. Because of the high homology between this receptor and the human β_3-adrenergic receptor, its unique pharmacological properties and fat cell specificity, we have defined this novel receptor as a β_{3A}(adipose)-adrenergic subtype.

The β_{3A}-adrenergic receptor exhibits several structural features common to G protein-coupled receptors. It contains seven regions of hydrophobic sequence that are presumed to represent transmembrane spanning domains (Figure 1). There are two putative sites of N-linked glycosylation (N-X-S/T) in the amino terminus and several serine and threonine residues in the COOH terminus and in the third intracellular loop that may serve as sites for regulation by protein kinases.

Furthermore, the β_{3A}-receptor contains several conserved amino acids at positions Asp^{80}, Asp^{114}, Asp^{131}, Cys^{107}, Cys^{186}, Cys^{192}, Cys^{193}, Ser^{209}, that have been demonstrated to play important roles in β-adrenergic receptor-ligand interactions and receptor activation by agonists (R.A.F. Dixon et al, Cold Spring Harbor Symp. Quant. Biol. 53, 487 (1988); J.C. Venter et al, Biochem. Pharmacol. 38, 1197 (1989); C.M. Fraser, J. Biol. Chem., 264, 9266 (1989); C.F. Strader, I.S. Sigal and R.A.F. Dixon, FASEB J. 3, 1825 (1989)).

Using a protocol for cotransfection of CHO cells (A 1.5 kb fragment was excised from pBluescript using Saci (present in the multiple cloning site of the vector) and BamHI and inserted into the Sac/BamH/ sites of PSVL (Pharmacie)). CHO-K1 cells were cotransfected with pSVL and pMSVneo (neomycin resistance plasmid) F.Z. Chung, C.D. Wang, P.C. Potter, J.C. Venter and C.M. Fraser, J. Biol. Chem. 263, 4052 (1988) using the aPO_4 precipitation technique. Stable transfectants were obtained by growth of the cells in culture medium containing Geneticin (500 µg/ml); colonies derived from single cells were isolated and expanded.

Because atypical β-adrenergic receptors in adipose tissue display low affinity for β-adrenergic antagonists, cell lines were screened for the expression of β-adrenergic receptors by measuring isoproterenol (10^{-6} M)-mediated increases in intracellular cAMP.), we obtained several clonal cell lines that permanently express the fat cell β receptor and chose one for additional pharmacological and biochemical characterization. Membranes from transfected CHO cells display saturable binding of the radioligand, [^{125}I]-iodocyanopindolol ([^{125}I]-ICYP) (Transfected cell membranes were prepared by lysis of cells in hypotonic solution containing 5 mM $NaPO_4$, pH 7.4, 2 mM $MgSO_4$ followed by centrifugation at 1000 X g for 5 minutes to remove intact cells and cell nuclei. The supernatant was centrifuged at 40,000 X g for 30 minutes to collect the membrane fraction.

Membrane associated β-adrenergic receptors (3-6 µg protein) were labeled with increasing concentrations of [^{125}I]-CYP in the presence and absence of 10 µM ICI 118,551 by incubation at 37°C for 30 minutes in Hank's buffer in a final volume of 250 µl. Incubations were terminated by filtration over Whatman GF/C glass fiber filters using a Brandel cell harveter. Scatchard analysis of saturation isotherms was performed to yield estimates of K_D (equilibrium dissociation constant for [^{125}I]-CYP) and B_{max} (total number of binding sites). The K_D value was utilized in computer analysis of competition displacement curves.) The calculated equilibrium dissociation constant (K_D) for [^{125}I]-ICYP binding is 1.3 ± 0.4 nM, a value significantly greater than K_D values for [^{125}I]-ICYP binding to β_1-(11pM) (13) and β_2-adrenergic receptors (30 pM) (D.A.

Robinson, thesis, State University of New York at Buffalo (1988)) but similar to that reported for [^{125}I]-ICYP binding to the β_3-adrenergic receptor (0.5 nM) (L.J. Emorine et al, Science 245, 1118 (1989)). The density of β-adrenergic receptors expressed in this cell line is 1100 ± 187 fmol/mg membrane protein.

Agonists produce dose-dependent increases in intracellular cAMP concentrations in transfected CHO cells with a rank order of potency of BRL 37344 > isoproterenol > norepinephrine > epinephrine > zinterol > tazolol (Figure 2, Table 1) (21). K_{act} values for BRL 37344 and isoproterenol-mediated increases in intracellular cAMP in transfected CHO cells are in very good agreement with the EC_{50} values for increases in lipolysis in brown adipocytes as described by Arch et al. (7); i.e., 1.3 and 1.7 nM for BRL 37344 and 4.0 and 8.0 nM for isoproterenol in transfected cells and brown adipocytes, respectively. The greater potency of norepinephrine as compared with epinephrine suggests that receptor activation in vivo is most likely mediated through sympathetic innervation. Antagonists (at 10^{-4} M concentrations) display an order of potency for inhibition of BRL 37344-induced cAMP accumulation in transfected CHO cells of propranolol (89% inhibition) > betaxolol (80% inhibition) > metoprolol (70% inhibition) > pindolol (61% inhibition) = 118,51 (60% inhibition) > alprenolol (52% inhibition) > atenolol (30% inhibition) (Figure 3).

In competition displacement studies (For competition displacement studies, membranes (containing 3-4 fmol [^{125}]-ICYP binding sites were incubated with [^{125}I]-CYP or [^{3}H]-CGP 12177 (~1 X K^D concentration)

plus a range of concentrations of competing ligands. Competition displacement curves were analyzed according to a mass action model for receptor-ligand interactions using a computerized interactive non-linear least squares curve-fitting program (GraphPAD INPLOT, San Diego, CA).

Competition displacement experiments were performed at least 3 times in triplicate. Triplicate values from each experiment were averaged and non-linear regression was performed on data averaged from all competition displacement curves for a given ligand), agonists display a rank order of potency of BRL 37344 (atypical β-adrenergic agonist) >> zinterol (β_2-adrenergic agonist) > tazolol (β_1-adrenergic agonist) > (-) isoproterenol > epinephrine > norepinephrine > (+) isoproterenol (Table 1).

The relative affinities of BRL 37344 and (-) isoproterenol for displacement of [^{3}H]-CGP 12177 are similar to those observed with [^{125}I]-ICYP. Antagonists display a rank order of potency of alprenolol > propranolol > ICI 118,551 (β_2-adrenergic selective) > betaxolol (β_1-adrenergic selective) (Table 1). The β_{3A}-adrenergic receptor exhibits a markedly lower affinity for classical β-adrenergic antagonists than either β_1 or β_2-adrenergic receptor subtypes.

The pharmacological properties of the β_{3A}-adrenergic receptor differ significantly from those reported by Emorine et al. (Science 245, page 1118 (1989) for a human β_3-receptor expressed in CHO cells. The rank order of agonist potency for inhibition of [^{125}I]-ICYP binding to the β_3-adrenergic receptor is BRL 37344 > norepinephrine > (-)a isoproterenol >> (+)

isoproterenol > epinephrine (11) as compared with BRL 37344 >> (-) isoproterenol > epinephrine > norepinephrine > (+) isoproterenol for the β_{3A}-adrenergic receptor.

The pharmacological profile of the β_{3A}-receptor does not agree with the human β_3-receptor.(L.J. Emorine et al, Science 245, 1118 (1989)). Also, it does not agree with any known tissue pharmacology, nor is it consistent with the pharmacological definition of a β-adrenergic receptor (isoproterenol more potent than either epinephrine or norepinephrine). In addition, most of the classical non-selective β-adrenergic antagonists do not inhibit [^{125}I]-ICYP binding to the β_3-adrenergic receptor (L.J. Emorine et al, Science 245, 1118 (1989)). Therefore, it is clear that there are substantial pharmacological differences between the fat cell β_{3A}-adrenergic receptor and the receptor described by Emorine et al. (L.J. Emorine et al, Science 245, 1118 (1989)).

The distribution of β-adrenergic receptor subtypes was determined as follows.

To further investigate the distribution of β-adrenergic receptor subtypes, poly $(A)^+$ RNAs from various tissues were isolated and fractionated on a formaldehyde-agarose gel (Figure 4) (Fifteen µg of poly $(A)^+$ RNA from rat IBAT, epididymal white adipose tissue, brain, heart, ileum, liver and lungs were electrophoresed in an agarose gel containing formaldehyde as described [H. Lehrach, D. Diamond, J.M. Wozney, H. Boedtker, Biochem. 16, 4743 (1977)] and transferred to Gene Screen Plus membranes (Dupont/New England Nuclear) by capillary blotting. Rat β_1-adrenergic receptor (Venter et al, unpublished), rat β-

adrenergic receptor (J. Gocayne et al, Proc. Natl. Acad. Sci. USA 84, 8296 (1987) and rat β_{3A}-adrenergic receptor cDNAs were labeled by random priming with [α-^{32}P]-dCTP (Dupont/New England Nuclear) to specific activities of ~ 1 X 10^9 dpm/µg DNA. RNA blots were hybridized overnight at 42°C in 45% formamide and 4X SSC (0.6M NaCl, 0.06M Na citrate) and then washed sequentially in a solution of 0.1X SSC, 0.1% sodium dodecyl sulfate at 55°C for 15 minutes followed by 60°C for 15 minutes.

Size estimates of RNA species were established by comparison with an RNA ladder. An mRNA species is detected at 3.1 kb with the β_1-adrenergic receptor probe, at 2.3 kb with the β_2-adrenergic receptor probe and at 2.3 kb with the β_{3A}-adrenergic receptor probe. Minor bands at 2.8, 3.8 and 4.6 kb are also detected with the β-$_{3A}$-adrenergic receptor probe. As a control, an oligo $(dT)_{12-18}$ probe was labeled with [γ^{32}P]-TTP using T4 polynucleotide kinase. Densitometric analysis of autoradiograms was performed with a high resolution densitometer. The values of the β-adrenergic receptor signals were normalized for the amount of poly $(A)^+$ RNA on the membranes with the corresponding oligo (dT) signal.

From the above experimentation, the distribution of β-adrenergic receptor subtypes in various tissues is as follows. β_1-adrenergic receptor mRNA is present in brown and white adipose tissue, brain, heart and lung. β_2-adrenergic receptor mRNA is also present in these tissues; however, with the exception of the lung, it is present at significantly lower levels. The β_{3A}-adrenergic receptor mRNA is abundant in brown adipose tissue, with no β_{3A}-receptor specific mRNA detectable

in brain, heart, ileum, liver or lung. White adipose tissue from rat (Figure 4) and human (data not shown) also contains an mRNA that hybridizes strongly with the β_{3A}-receptor cDNA probe.

It has been difficult to quantitate the atypical β-adrenergic receptor in adipose tissue since radiolabeled antagonists commonly used display significantly (up to 100-fold) greater affinities for β_1- and β_2-adrenergic receptor subtypes than for the atypical β-adrenergic receptor. However, under identical conditions using probes of similar specific activities, it was estimated that the β_{3A}-receptor mRNA is present in a 5-fold and 4-fold excess over β_1-receptor mRNA in brown and white adipose tissue, respectively, whereas β_2-receptor mRNA is virtually undetectable (data not shown). Thus, the relative amounts of receptor subtype-specific mRNA species suggest that the β_{3A}-adrenergic receptor, which is presumed to mediate lipolysis (U.R.S. Arch et al, Nature (London) 309, 163 (1984)), is the predominant β-receptor in adipose tissue.

Further to the above experimentation to determine receptor distribution and its effects on obesity, the folloiwing relates to normal vs. abnormalities in brown adipose tissue.

Numerous investigations have reported abnormalities in brown adipose tissue of heredity obese animals (J. Himms-Hagen, Prog. Lip. Res. 28, 67 (1989)). In both obese (ob/ob) mice and (fa/fa) Zucker rats, the thermogenic response of brown adipose tissue to sympathetic stimulation is decreased as compared with lean controls (F. Assimacopoulos-Jeannet, J.P. Giacobino, J. Seydoux, L. Girardier, B. Jeanrenaud,

Endocrinol. 110, 439 (1982); A. Marette, A. Geloen, A. Collett and J. Bukowiecki, Am. J. Physiol. 258, E320 (1990)). In obese (fa/fa) Zucker rats, β-adrenergic stimulation of adenylate cyclase is also reduced (P. Muzzin, J.P. Revelli, D. Ricquier, M..K. Meier, F. Assimacopoulos-Jeannet, J.P. Giacobino, Biochem. J. 261, 721 (1989)).

Since it is possible that the decrease in tissue responsiveness may reflect changes in β-adrenergic receptor expression, we examined the levels of β-adrenergic receptor mRNA in obese (fa/fa) Zucker rats and lean control (Fa/Fa) animals. Male obese (fa/fa) Zuker and lean (Fa/Fa) control rats (9 weeks old) was isolated and Northern blot analysis was performed. The Student's unpaired t-test was used to determine statistical significance.) . As shown in Figure 5, β_1 and β_2-adrenergic receptor mRNA levels are unchanged in brown and white fat of obese rats. In contrast, the level of β_{3A}-adrenergic receptor mRNA is decreased by 60% and 71%, respectively, in brown and white fat of obese animals as compared with lean controls. The selective decrease in β_{3A}-adrenergic receptor could account for the observed catecholamine resistance of obese animals (P. Muzzin et al, Biochem. J. 261, 721 (1989)).

Accordingly, the β_{3A}-adrenergic receptor according to the present invention, which is expressed in adipose tissue differs significantly from β_1-, β_2-, and β_3-adrenergic receptors previously described (C.A. Machida et al., J. Biol. hem. 265, 12960 (1990); F.Z. Chung et al., FEBS Lett. 211, 200 (1987)). Identification of this unique β-adrenergic receptor in adipose tissue of rats and humans and the demonstration that receptor

mRNA levels are markedly reduced in an animal model of genetic obesity provide a basis for detection and regulation of this receptor in physiological and pathological conditions in rodents and man.

As is clear from the above experimental data and comparisons between the present receptor polypeptide and that of the prior art, the present β_{3A}-adrenergic receptor and its applications are a significant advancement over the prior art. The advancements are very useful to treat or study obesity.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept and therefore such adaptations are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description only and not of limitation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: J. Craig Venter et al

(ii) TITLE OF INVENTION: A FAT CELL SPECIFIC β-ADRENERGIC RECEPTOR

(iii) NUMBER OF SEQUENCES: 1

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Polypeptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Pro Trp Pro His Lys Asn Gly Ser Leu Ala Phe Trp Ser Asp
1 5 10 15
Ala Pro Thr Leu Asp Pro Ser Ala Ala Asn Thr Ser Gly Leu Pro Gly
20 25 30
Val Pro Trp Ala Ala Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Thr
35 40 45
Val Gly Gly Asn Leu Leu Val Ile Thr Ala Ile Ala Arg Thr Pro Arg
50 55 60
Leu Gln Thr Ile Thr Asn Val Phe Val Thr Ser Leu Ala Thr Ala Asp
65 70 75 80
Leu Val Val Gly Leu Leu Val Met Pro Pro Gly Ala Thr Leu Ala Leu
85 90 95
Thr Gly His Trp Pro Leu Gly Ala Thr Gly Cys Glu Leu Trp Thr Ser
100 105 110
Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu
115 120 125
Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Thr
130 135 140
Leu Val Thr Lys Arg Arg Ala Arg Ala Ala Val Val Leu Val Trp Ile
145 150 155 160
Val Ser Ala Thr Val Ser Phe Ala Pro Ile Met Ser Gln Trp Trp Arg
165 170 175
Val Gly Ala Asp Ala Glu Ala Gln Glu Cys His Ser Asn Pro Arg Cys
180 185 190
Cys Ser Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser Ser Ser Val
195 200 205
Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr Ala Arg Val
210 215 220
Phe Val Val Ala Lys Arg Gln Arg Arg Phe Val Arg Arg Glu Leu Gly
225 230 235 240
Arg Phe Pro Pro Glu Glu Ser Pro Arg Ser Pro Ser Arg Ser Pro Ser
245 250 255
Pro Ala Thr Val Gly Thr Pro Thr Ala Ser Asp Gly Val Pro Ser Cys
260 265 270
Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Gly Glu His Arg Ala Leu
275 280 285
Arg Thr Leu Gly Leu Ile Met Gly Ile Phe Ser Leu Cys Trp Leu Pro
290 295 300
Phe Phe Leu Ala Asn Val Leu Arg Ala Leu Val Gly Pro Ser Leu Val
305 310 315 320
Pro Ser Gly Val Phe Ile Ala Leu Asn Trp Leu Gly Tyr Ala Asn Ser
325 330 335
Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Asp Ala
340 345 350
Phe Arg Arg Leu Leu Cys Ser Tyr Gly Gly Arg Gly Pro Glu Glu Pro
355 360 365
Arg Val Val Thr Phe Pro Ala Ser Pro Val Ala Ser Arg Gln Asn Ser
370 375 380
Pro Leu Asn Arg Phe Asp Gly Tyr Glu Gly Glu Arg Pro Phe Pro Thr
385 390 395 400

Claims

1. A highly accurate and sensitive specific β-adrenergic receptor that mediates lipolysis.

2. A β-adrenergic receptor according to claim 1 having a polypeptide sequence according to SEQ ID NO:1.

3. A cloned cell encoding for a specific fat cell β-adrenergic receptor according to claim 1.

4. A cloned cell according to claim 3 which is obtained by cotransfection of CHO cells.

5. A cloned cell according to claim 4 which produces an adrenergic receptor having the sequence according to SEQ ID NO:1.

6. A diagnostic test for determining decreased levels of fat cell β-adrenergic receptors that mediate lipolysis according to claim 1 comprising detecting the level of a β-adrenergic receptor that mediates lipolysis and comparing it to a lean control host level in order to diagnosis obesity caused by less active lipolysis.

7. A diagnostic test according to claim 6 for determining decreased levels of a β-adrenergic receptor polypeptide having a sequence according to SEQ ID NO:1.

```
Human ß2    1            M          gqpGNgSaFLL    APNrShAPdHDvTQqRDEvWVVGMgIvMSLI
Rat ß2      1            MEP          hGNdSdFLL    APNgSrAPgHDiTQeRDEaWVVGMaIlMSvI
Rat ß1      1  MGAGaLaLGASEPcNLSSAAPLPDGAATAARLLVlASPPASLLPPASEgsaPLSQQWTAGMGLLlALI
Human ß1    1  MGAGvLvLGASEPgNLSSAAPLPDGAATAARLLVpASPPASLLPPASEspePLSQQWTAGMGLLmALI
Human ß3     1          MAPWPHENSSLAPWPD     lPTLaPntANTSGLP      GVPWeAALAG ALL ALA
Rat ß3      1          MAPWPHkNgSLAfWsD     aPTLdPsaANTSGLP      GVPWaAALAG ALL ALA
                                 *                  *                            I

Human ß2   44  VLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMGLAVVPFGAaHILMKMWtFGNFWCEFWTS
Rat ß2     44  VLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMGLAVVPFGAsHILMKMWnFGNFWCEFWTS
Rat ß1     69  VLLIVvGNVLVIVAIAKTPRLQTLTNLFIMSLASADLVMGLLVVPFGATIVVWGRWEYGSFFCELWTS
Human ß1   69  VLLIVaGNVLVIVAIAKTPRLQTLTNLFIMSLASADLVMGLLVVPFGATIVVWGRWEYGSFFCELWTS
Human ß3   48  VLaTVGGNLLVIVAIAwTPRLQTmTNVFVTSLAaADLVMGLLVVPPaATLALTGHWPLGATGCELWTS
Rat ß3     48     TVGGNLLVItAIArTPRLQTiTNVFVTSLAtADLVvGLLVmPPgATLALTGHWPLGATGCELWTS
                                                       II

Human ß2  112  IDVLCVTASIETLCVIAVDRYfAITSPFKsQSLLTKNKARViILMVWIVSGLTSFLPIQMHWYR ATH
Rat ß2    112  IDVLCVTASIETLCVIAVDRYvAITSPFKYQSLLTKNKARVvILMVWIVSGLTSFLPIQMHWYR ATH
Rat ß1    137  VDVLCVTASIETLCVIALDRYLAITlPFRYQSLLTRARARaLVCTVWAISALVSFLPILMHWWRAESD
Human ß1  137  VDVLCVTASIETLCVIALDRYLAITsPFRYQSLLTRARARgLVCTVWAISALVSFLPILMHWWRAESD
Human ß3  116  VDVLCVTASIETLCALAVDRYLAVTNPLRYGaLVTKRcARtAVVLVWvVSAaVSFAPIMSQWWRVGAD
Rat ß3    113  VDVLCVTASIETLCALAVDRYLAVTNPLRYGtLVTKRrARaAVVLVWiVSAtVSFAPIMSQWWRVGAD
                     III                                          IV
```

FIG. 1A

```
Human ß2  179 qeAInCYAnETCCDFFTNQAYAIASSIVSFYVPLViMVFVYSRVFQeAKRQLQKIDKSEGRF
Rat ß2    179 kqAIdCYAkETCCDFFTNQAYAIASSIVSFYVPLVvMVFVYSRVFQvAKRQLQKIDKSEGRF
Rat ß1    204 DEARRCYNDPKCCDFVTNRAYAIASSVVSFYVPLCIMAFVYLRVFREAQKQVKKIDSCERRFLtGPpR
Human ß1  204 DEARRCYNDPKCCDFVTNRAYAIASSVVSFYVPLCIMAFVYLRVFREAQKQVKKIDSCERRFLgGPaR
Human ß3  184 AEAQRCHSNPRCCaFASNMPYvLLSSSVSFYLPLLVMLFVYARVFVVAtRQlRllRgELGRF PPEES
Rat ß3    181 AEAQeCHSNPRCCsFASNMPYaLLSSSVSFYLPLLVMLFVYARVFVVAkRQrRfvRrELGRF PPEES
                                  [redacted]
                                       V
Human ß2  241                QVEQHvQNLSQVEQDGRtGHGLRrS     SnFCLKEHKALKTLGIIMGTFTLCWLP
Rat ß2    241                   HaQNLSQVEQDGRSGHGLRSS     SkFCLKEHKALKTLGIIMGTFTLCWLP
Rat ß1    272 PPSPaPSP      sPGPPRPA     dSLANGRSSKRRPSRLVALREQKALKTLGIIMGVFTLCWLP
Human ß1  272 PPSPsPSPvpAPAPpPGPPRPAaaaatAPLANGRAgKRRPSRLVALREQKALKTLGIIMGVFTLCWLP
Human ß3  251 PPaPS  RSlAPAP          VGTcAPpeGVPACGRRPARLLPLREHRALcTLGLIMGtFTLCWLP
Rat ß3    248 PrsPS  RSpsPA          tVGTptasdGVPsCGRRPARLLPLgEHRALrTLGLIMGiFsLCWLP
                                                                  [redacted]
                                                                       VI
Human ß2  289 FFIVN IVHVIqaNLIrKEVYILLNWiGYVNSgFNPLIYCRSPDFRIAFQELLC       LRR  SS
Rat ß2    289 FFIVN IVHVIrdNLIPKEVYILLNWLGYVNSAFNPLIYCRSPDFRIAFQELLC       LRR  SS
Rat ß1    329 FFLAN  VKAFHRdLVPDRLFVFFNWLGYANSAFNPIIYCRSPDFRKAFQrLLCCARRAAcRRR AaH
Human ß1  340 FFLAN  VKAFHReLVPDRLFVFFNWLGYANSAFNPIIYCRSPDFRKAFQgLLCCARRAA RRRhAtH
Human ß3  308 FFLANVLRALgGPSLVPgpaFlALNWLGYANSAFNPLIYCRSPDFRsAFRRLLC     rcGRR
Rat ß3    305 FFLANVLRALvGPSLVPsgvFiALNWLGYANSAFNPLIYCRSPDFRdAFRRLLC     syGgR
          [redacted]  [redacted]
                                       VII
```

FIG. 1B

```
Human  ß2   347 lKaYGNGYSSN     gnTGEQ  YhveQEKENkLLCEdlPGtEdFVghQGTVPSdnIDSQGRNCsTN
Rat  ß2     347 sKtYGNGYSSNsnGrtdyTGEQSaYqlgQEKENeLLCEeaPGmEGFVncQGTVPSlSIDSQGRNCnTN
Rat  ß1     395 GDRPRASGCLARaGPPPSPGApSDDDDDD aGATPPARLLEPWAGCNGGttTVDSDSSLDEPgRqGFs
Human  ß1   406 GDRPRASGCLARPGPPPSPGAaSDDDDDDvvGATPPARLLEPWAGCNGG AaaDSDSSLDEPcRpGFa
Human  ß3   367   lPpEPcaaARPalFPS                GvPA        arsspAqprlcqrLDgvtgaegp
Rat  ß3     364   gPeEP    RvvtFPaspvasrqnsplnrfdGyegerpfpt


Human  ß2   409 DSlL
Rat  ß2     404 DSpL
Rat  ß1     457 SESKV
Human  ß1   468 SESKV
Human  ß3   402 a
Rat  ß3
```

FIG. 1C

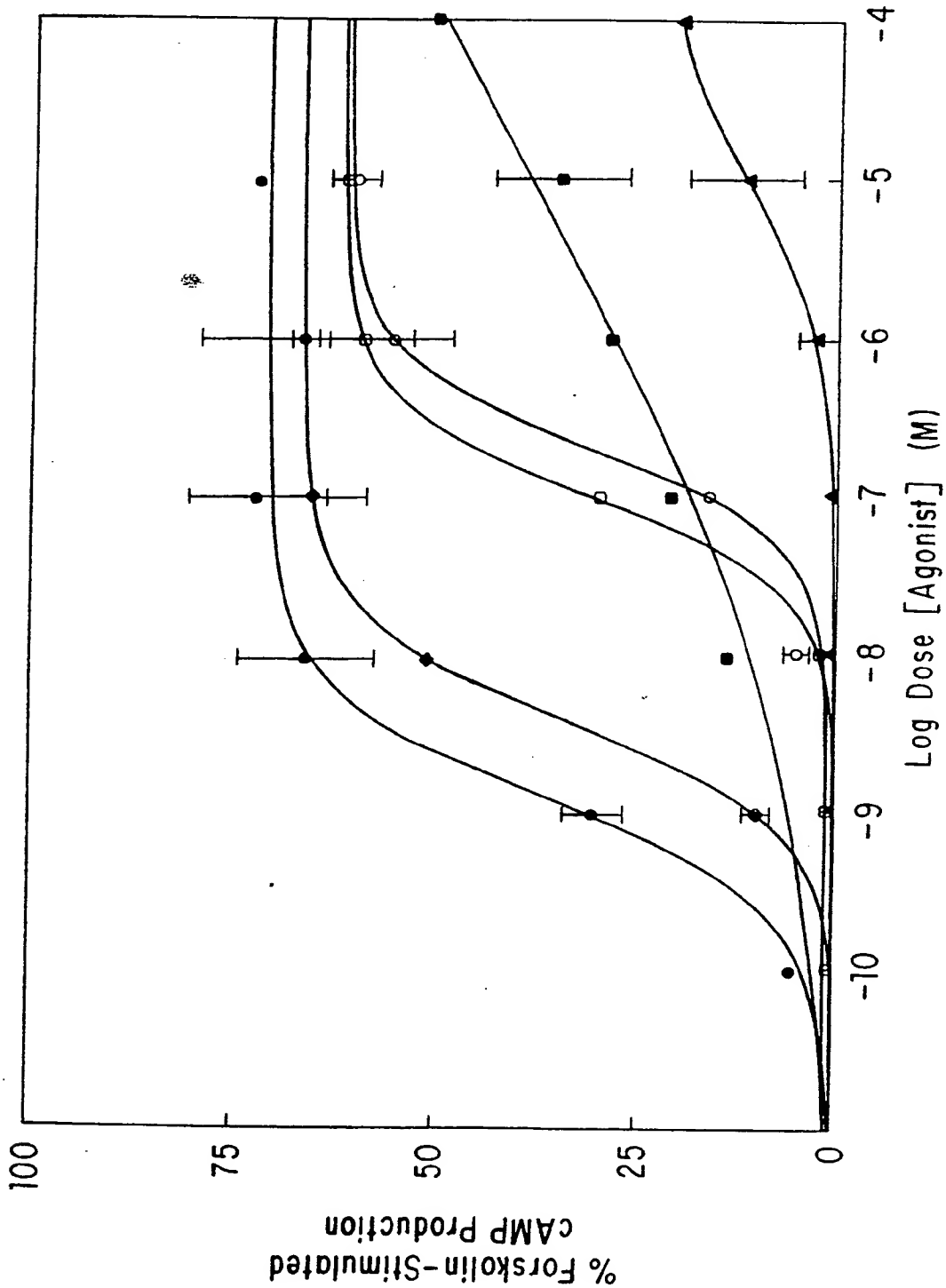
% Forskolin-Stimulated cAMP Production
100
75
50
25
0
-4
-5
-6
-7
-8
-9
-10
Log Dose [Agonist] (M)

FIG. 2

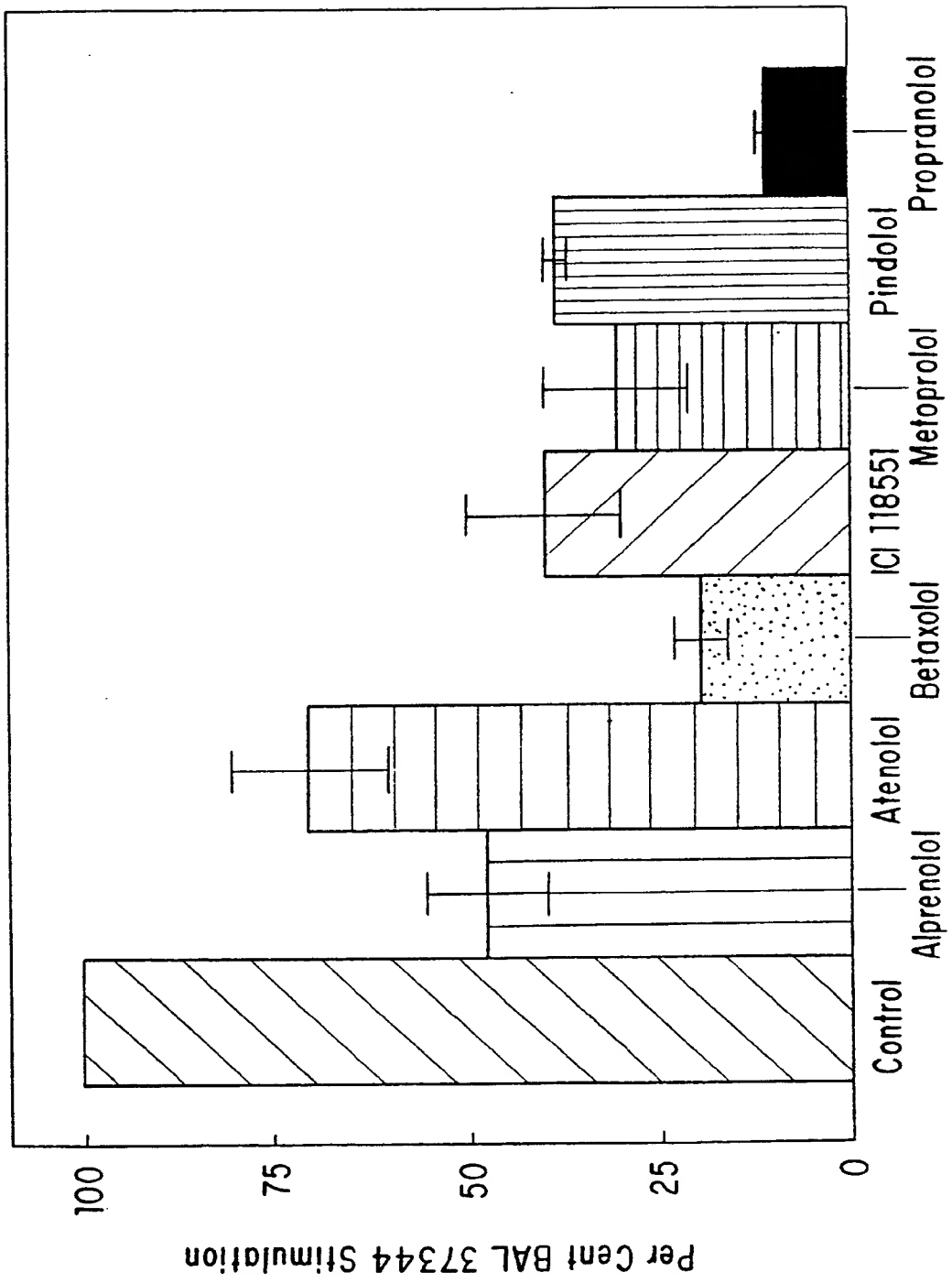
Per Cent BAL 37344 Stimulation
100
75
50
25
0
Control
Alprenolol
Atenolol
Betaxolol
ICI 118551
Metoprolol
Pindolol
Propranolol

FIG. 3

FIG. 4A

FIG. 4B

FIG. 4C

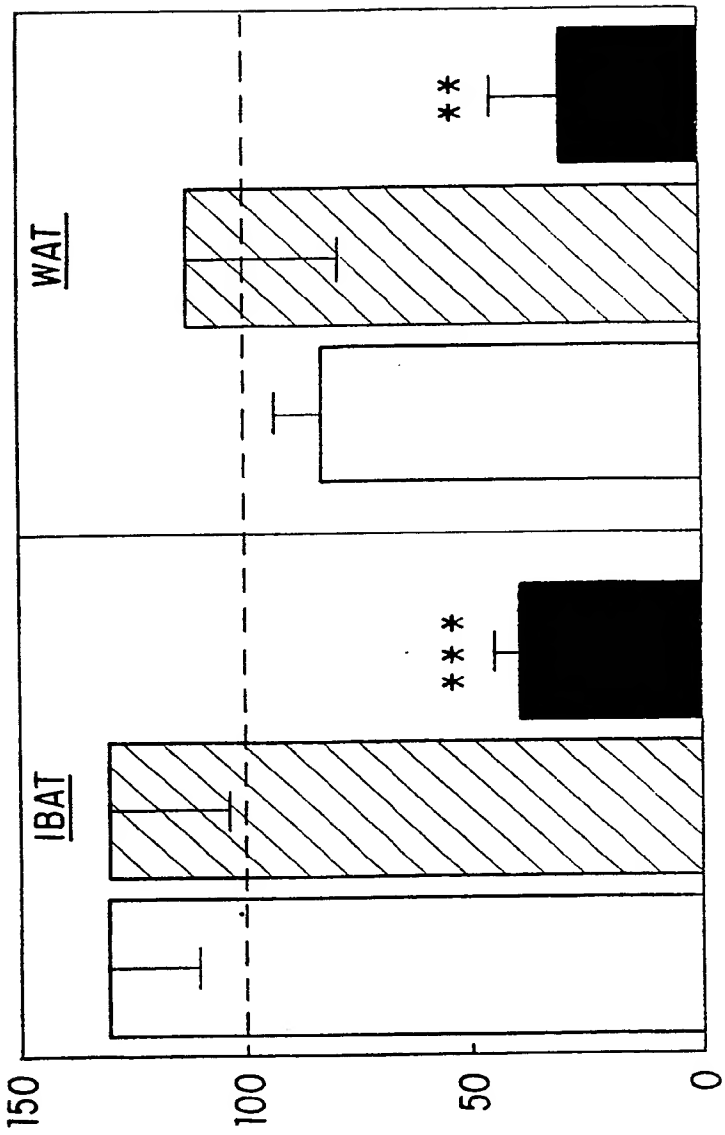

FIG. 5

```
Met Ala Pro Trp Pro His Lys Asn Gly Ser Leu Ala Phe Trp Ser Asp
 1               5                  10                  15
Ala Pro Thr Leu Asp Pro Ser Ala Ala Asn Thr Ser Gly Leu Pro Gly
            20                  25                  30
Val Pro Trp Ala Ala Ala Leu Ala Gly Ala Leu Leu Ala Leu Ala Thr
        35                  40                  45
Val Gly Gly Asn Leu Leu Val Ile Thr Ala Ile Ala Arg Thr Pro Arg
    50                  55                  60
Leu Gln Thr Ile Thr Asn Val Phe Val Thr Ser Leu Ala Thr Ala Asp
65                  70                  75                  80
Leu Val Val Gly Leu Leu Val Met Pro Pro Gly Ala Thr Leu Ala Leu
                85                  90                  95
Thr Gly His Trp Pro Leu Gly Ala Thr Gly Cys Glu Leu Trp Thr Ser
            100                 105                 110
Val Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Ala Leu
        115                 120                 125
Ala Val Asp Arg Tyr Leu Ala Val Thr Asn Pro Leu Arg Tyr Gly Thr
    130                 135                 140
Leu Val Thr Lys Arg Arg Ala Arg Ala Ala Val Val Leu Val Trp Ile
145                 150                 155                 160
Val Ser Ala Thr Val Ser Phe Ala Pro Ile Met Ser Gln Trp Trp Arg
                165                 170                 175
Val Gly Ala Asp Ala Glu Ala Gln Glu Cys His Ser Asn Pro Arg Cys
            180                 185                 190
Cys Ser Phe Ala Ser Asn Met Pro Tyr Ala Leu Leu Ser Ser Ser Val
        195                 200                 205
```

FIG. 6A

```
Ser Phe Tyr Leu Pro Leu Leu Val Met Leu Phe Val Tyr Ala Arg Val
    210                 215                 220
Phe Val Val Ala Lys Arg Gln Arg Arg Phe Val Arg Arg Glu Leu Gly
225                 230                 235                 240
Arg Phe Pro Pro Glu Glu Ser Pro Arg Ser Pro Ser Arg Ser Pro Ser
                245                 250                 255
Pro Ala Thr Val Gly Thr Pro Thr Ala Ser Asp Gly Val Pro Ser Cys
            260                 265                 270
Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Gly Glu His Arg Ala Leu
        275                 280                 285
Arg Thr Leu Gly Leu Ile Met Gly Ile Phe Ser Leu Cys Trp Leu Pro
    290                 295                 300
Phe Phe Leu Ala Asn Val Leu Arg Ala Leu Val Gly Pro Ser Leu Val
305                 310                 315                 320
Pro Ser Gly Val Phe Ile Ala Leu Asn Trp Leu Gly Tyr Ala Asn Ser
                325                 330                 335
Ala Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Asp Ala
            340                 345                 350
Phe Arg Arg Leu Leu Cys Ser Tyr Gly Gly Arg Gly Pro Glu Glu Pro
    355                 360                 365
Arg Val Val Thr Phe Pro Ala Ser Pro Val Ala Ser Arg Gln Asn Ser
370                 375                 380                 385
Pro Leu Asn Arg Phe Asp Gly Tyr Glu Gly Glu Arg Pro Phe Pro Thr
                390                 395                 400
```

FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09379

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 13/00; C12N 5/10; C12Q 1/68
US CL :530/350; 435/240.2, 6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/240.2, 6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

cas online, aps, medline, biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
x — y	Science, Volume 245, issued 08 September 1989, L. J. Emorine et al., "Molecular Characterization of the Human Beta-3-Adrenergic Receptor", pages 1118-1121, entire document.	1,3-4 —— 2,5-7
y	Nature, Volume 309, issued 10 May 1984, J. R. S. Arch et al., "Atypical Beta-adrenoceptor on Brown Adipocytes as Target for Anti-Obesity Drugs", pages 163-165, entire document.	2,5-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 January 1993	Date of mailing of the international search report 26 JAN 1993
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer KENNETH R. HORLICK Telephone No. (703) 308-0196

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